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# Cell-specific perforation using functionalized plasmonic nanoparticles and near-infrared femtosecond laser

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**Introduction:** Gold nanoparticles (AuNPs) have found numerous applications in nanomedicine, in view of their robustness, ease of functionalization and low toxicity<sup>[1],[2]</sup>. Our group has demonstrated that near-infrared (NIR) femtosecond (fs) laser excitation of 100 nm citrate-capped AuNPs enables high cell membrane perforation, while maintaining high cell viability and enabling transfection of DNA plasmids into cancer cells<sup>[3]</sup>. Such laser irradiation of AuNPs minimizes the heat absorption from AuNPs and biological tissues since they absorb energy very weakly in the NIR range<sup>[4],[5]</sup>. The amplification of the electromagnetic field around the irradiated AuNP can cause the generation of nanoplasma leading to a nanobubble, thus inducing cell perforation without AuNP fragmentation<sup>[3],[6]–[8]</sup>.

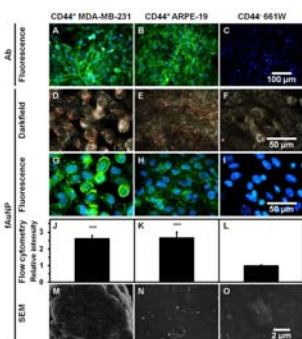
Since selective targeting of diseased cells can increase therapeutic efficacy and limit off-target adverse effects, the next step for selective cell optoporation is to develop stable AuNPs which labeled targeted cells without affecting surrounding non-targeted cells. Here the receptor CD44 strongly expressed by cancer cells was used as a model for selective targeting and optoporation.

**Materials:** Monoclonal anti-CD44 antibodies (Abs) from abcam. Orthopyridyl-disulfide-poly(ethylene glycol) (5kDa)-*N*-hydroxysuccinimide (OPSS-PEG-NHS) and HS-PEG (5kDa) from Nanocs. Citrate-capped AuNPs (50 µg/mL, 100 nm in diameter) from Nanopartz. HS-PEG (2kDa), PBS, Lucifer Yellow (LY) and DAPI from Sigma-Aldrich. Tetramethylrhodamine-wheat germ agglutinin (WGA) and Alexa 488-Abs from Life Technologies.

**Methods:** Abs were conjugated to OPSS-PEG-NHS (OPSS-PEG-Ab). AuNPs were functionalized with 0.01 OPSS-PEG-Ab/nm<sup>2</sup> and 5 µM HS-PEG<sup>[9]</sup>. The stability of functionalized AuNPs (fAuNPs) was evaluated by UV-visible spectroscopy and zeta potential measurements. Cells were incubated for 3 h with 8 µg/mL fAuNPs, washed with PBS, incubated with LY and treated with a 45 fs laser at 800 nm (Spitfire, Spectra Physics). Two hours after the laser treatment, the cells were washed with PBS, fixed and stained with DAPI.

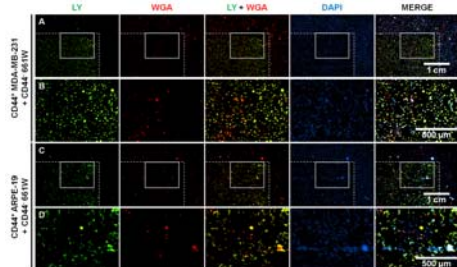
**Results and Discussion:** The fAuNPs with HS-PEG (5kDa) were colloiddally stable in cell culture medium containing serum proteins, while fAuNPs with HS-PEG (2kDa) were unstable.

Selective targeting with stable fAuNPs was confirmed by immunofluorescence, darkfield and fluorescence imaging, flow cytometry and SEM on targeted CD44<sup>+</sup> human cells (MDA-MB-231 breast cancer and ARPE-19 retinal pigmented epithelium) and on non-targeted CD44<sup>-</sup> mouse 661W photoreceptors (Fig. 1). The fAuNPs attached mostly as single particles 115 times more to targeted cells than to non-targeted cells.

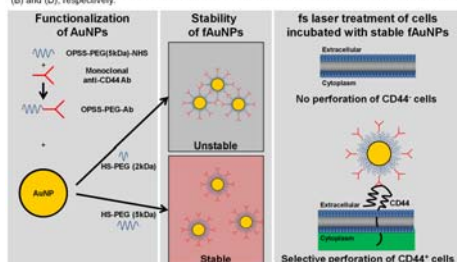


**Fig. 1. Selective labeling of targeted cells incubated with stable fAuNPs.**  
(A-C) Immunofluorescence of CD44 receptors in CD44<sup>+</sup> MDA-MB-231, CD44<sup>+</sup> ARPE-19 and CD44<sup>-</sup> 661W cells. Darkfield (D-F) and fluorescence (G-I) imaging of cells with fAuNPs. Anti-CD44 Abs were detected with Alexa Fluor 488 conjugated to goat anti-rat IgG Abs and cell nuclei were stained with DAPI (A-C, G-I). (J-L) Mean forward scattering intensity of cells with fAuNPs was recorded by flow cytometry and normalized to cells without fAuNPs for each cell type (CTL). Results are expressed as means  $\pm$  standard deviation. Statistically significant differences are indicated by \*\*\* $p$  < 0.001 in comparison to CTL. (M-O) Scanning electron microscopy (SEM) of cells with fAuNPs.

Selective optoporation of targeted cells was demonstrated with stable fAuNPs enhancing fs laser without affecting surrounding non-targeted cells (Fig. 2 and 3). Perforated cells in the laser-irradiated area were mainly targeted cells which accumulated LY, while untreated cells remained non-perforated<sup>[10]</sup>.



**Fig. 2. Selective optoporation of targeted cells without affecting surrounding non-targeted cells.**  
Fluorescence imaging of targeted CD44<sup>+</sup> MDA-MB-231 (A,B) and CD44<sup>+</sup> ARPE-19 (C,D) cells in co-culture with CD44<sup>-</sup> 661W cells stained with WGA incubated with stable fAuNPs. LY was added before laser treatment (60 mJ/cm<sup>2</sup>, 500 Hz) and cell nuclei were stained with DAPI. The laser irradiated area is shown in the left box delimited with dashed line. The magnified central area from (A) and (C) are shown in (B) and (D), respectively.



**Fig. 3. Schematic representation of selective optoporation with fs laser and stable fAuNPs.**

**Conclusion:** The proposed novel highly versatile treatment paradigm can be adapted to target and perforate other cell populations by adapting to desired biomarkers. Since living biological tissues absorb energy very weakly in the NIR range, the developed non-invasive tool may provide a safe, cost-effective clinically relevant approach to ablate pathologically deregulated cells and limit complications associated with surgical interventions.

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